



## Contribution of the flexible incudo-malleal joint to middle-ear sound transmission under static pressure loads

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**Abstract:** The incudo-malleal joint (IMJ) in the human middle ear is a true diarthrodial joint and it has been known that the flexibility of this joint does not contribute to better middle-ear sound transmission. Previous studies have proposed that a gliding motion between the malleus and the incus at this joint prevents the transmission of large displacements of the malleus to the incus and stapes and thus contributes to the protection of the inner ear as an immediate response against large static pressure changes. However, dynamic behavior of this joint under static pressure changes has not been fully revealed. In this study, effects of the flexibility of the IMJ on middle-ear sound transmission under static pressure difference between the middle-ear cavity and the environment were investigated. Experiments were performed in human cadaveric temporal bones with static pressures in the range of  $\pm 2$  kPa being applied to the ear canal (relative to middle-ear cavity). Vibrational motions of the umbo and the stapes footplate center in response to acoustic stimulation (0.2-8 kHz) were measured using a 3D-Laser Doppler vibrometer for (1) the natural IMJ and (2) the IMJ with experimentally-reduced flexibility. With the natural condition of the IMJ, vibrations of the umbo and the stapes footplate center under static pressure loads were attenuated at low frequencies below the middle-ear resonance frequency as observed in previous studies. After the flexibility of the IMJ was reduced, additional attenuations of vibrational motion were observed for the umbo under positive static pressures in the ear canal (EC) and the stapes footplate center under both positive and negative static EC pressures. The additional attenuation of vibration reached 4.7 dB for the umbo under positive static EC pressures and the stapes footplate center under negative EC pressures, and 7.11 dB for the stapes footplate center under positive EC pressures. The results of this study indicate an adaptive mechanism of the flexible IMJ in the human middle ear to changes of static EC pressure by reducing the attenuation of the middle-ear sound transmission. Such results are expected to be used for diagnosis of the IMJ stiffening and to be applied to design of middle-ear prostheses.

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## Research Paper

# Contribution of the flexible incudo-malleal joint to middle-ear sound transmission under static pressure loads

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## ABSTRACT

The incudo-malleal joint (IMJ) in the human middle ear is a true diarthrodial joint and it has been known that the flexibility of this joint does not contribute to better middle-ear sound transmission. Previous studies have proposed that a gliding motion between the malleus and the incus at this joint prevents the transmission of large displacements of the malleus to the incus and stapes and thus contributes to the protection of the inner ear as an immediate response against large static pressure changes. However, dynamic behavior of this joint under static pressure changes has not been fully revealed. In this study, effects of the flexibility of the IMJ on middle-ear sound transmission under static pressure difference between the middle-ear cavity and the environment were investigated. Experiments were performed in human cadaveric temporal bones with static pressures in the range of  $\pm 2$  kPa being applied to the ear canal (relative to middle-ear cavity). Vibrational motions of the umbo and the stapes footplate center in response to acoustic stimulation (0.2–8 kHz) were measured using a 3D-Laser Doppler vibrometer for (1) the natural IMJ and (2) the IMJ with experimentally-reduced flexibility. With the natural condition of the IMJ, vibrations of the umbo and the stapes footplate center under static pressure loads were attenuated at low frequencies below the middle-ear resonance frequency as observed in previous studies. After the flexibility of the IMJ was reduced, additional attenuations of vibrational motion were observed for the umbo under positive static pressures in the ear canal (EC) and the stapes footplate center under both positive and negative static EC pressures. The additional attenuation of vibration reached 4–7 dB for the umbo under positive static EC pressures and the stapes footplate center under negative EC pressures, and 7–11 dB for the stapes footplate center under positive EC pressures. The results of this study indicate an adaptive mechanism of the flexible IMJ in the human middle ear to changes of static EC pressure by reducing the attenuation of the middle-ear sound transmission. Such results are expected to be used for diagnosis of the IMJ stiffening and to be applied to design of middle-ear prostheses.

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**Abbreviations:** AEC, artificial ear canal; AF, after fixation of the incudo-malleal joint; ASTM, American Society for Testing and Materials; BF, before fixation of the incudo-malleal joint; DAQ, data acquisition device; EC, ear canal; IMJ, incudo-malleal joint; LDV, Laser Doppler vibrometer; SFC, stapes footplate center; TF, transfer function.

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## 1. Introduction

The human middle ear cavity is an isolated space which is occasionally ventilated, and thereby pressure-regulated, by the opening of the eustachian tube. The eustachian tube passively opens at a pressure difference (between the external ear canal the middle-ear cavity) of 2 kPa, which corresponds to approximately 122-meter altitude change (Mirza and Richardson, 2005). While this ventilation mechanism is effective in protecting fragile ear structures from damage caused by environmental static pressure changes, the re-

sponse is time-delayed. In case of an eustachian tube malfunction, otitis media or when the environmental pressure changes rapidly, e.g. in an airplane, the middle-ear cavity is not pressure-equalized to the exterior anymore.

The incudo-malleal joint (IMJ) in the human middle ear is a true diarthrodial joint of a complex saddle-shape and is encapsulated by elastic or viscoelastic boundaries (Kirikae, 1960; Harty, 1964; Etholm and Belal, 1974; Marquet, 1981; Sim and Puria, 2008). The deformability of this joint allows for relative motion between the malleus and the incus (Hüttenbrink, 1988; Willi et al., 2002; Offergeld et al., 2007; Dobrev et al., 2016). Puria and Steele (2010) hypothesized that the flexibility of the IMJ may contribute to middle-ear sound transmission at high frequencies.

However, measurements by Gerig et al. (2015) showed that the middle-ear sound transmission above 2 kHz is amplified by reducing the flexibility of the IMJ, indicating that the flexibility of the IMJ in the human middle ear does not contribute to better sound transmission through the middle ear. Instead, studies by Hüttenbrink (1988, 1997) and Ihrle et al. (2016) proposed that the flexibility of the IMJ in humans may perform a protective function as an immediate response to environmental static pressure changes. In detail, inward (by positive external pressures) or outward (by negative external pressures) movement of the malleus in responses to static pressure changes causes a gliding movement between the malleus and the incus at the IMJ, preventing deep intrusion of the stapes in piston-like motion direction, which can lead to damage of inner ear structures. Thus the flexible IMJ fulfills a protective role for inner ear structures against large displacements of the tympanic membrane and the umbo caused by changes of external static pressure and thereby is considered valuable in lessening the risk of hearing loss under sudden environmental changes.

Several studies (e.g., Murakami et al. 1997; Gan et al., 2006; Homma et al., 2010) found that pressure differences between the middle-ear cavity and the external ear canal affect middle-ear sound transmission. They observed that vibrations of the umbo and the stapes in human cadaveric temporal bones were considerably attenuated at lower frequencies under these conditions. If the protective function of the flexible IMJ reduces displacements of the stapes under static pressure loads, the flexibility of the IMJ will contribute to reduction of the stress level on the stapes annular ligament and thus improvement of middle-ear sound transmission under static pressure loads. However, it has not been experimentally shown how the flexibility of the IMJ in humans contributes to the attenuation of the middle-ear sound transmission under the static pressure difference across the tympanic membrane.

In the present study, we aimed to investigate the influence of the flexible nature of the IMJ on middle-ear sound transmission with static pressure imbalance between the middle-ear cavity and the external ear canal, pointing at a possible adaptation mechanism of the flexible IMJ for hearing perception, i.e., minimization of hearing degradation under changes of static pressure, in addition to the previously proposed protective mechanism of the IMJ.

## 2. Methods

This study was approved by the Swiss Ethic Commission of Canton Zürich with the identification number KEK-ZH-Nr. 2014-0544.

### 2.1. Temporal bone preparation

Results for four frozen human cadaveric temporal bones are reported in this article (1 female, 3 males, 58–77 years). They were harvested by the provider (Science Care, Phoenix, USA) between

48–72 h after death and were frozen immediately. A wide mastoidectomy with posterior tympanotomy was performed and the tectum surrounding the IMJ was removed in order to gain a broad access. The ear canal was drilled down to 2–3 mm from the tympanic annulus, and a plastic artificial ear canal (AEC) was glued to the remaining bony rim of the tympanic membrane, making a chamber of approximately 0.5-ml volume (Sim et al., 2010, 2012). The interface between the AEC and the bony rim was further reinforced with an epoxy glue (Rapid, Araldite) to prevent static pressure leaks. The AEC allowed for accessibility to the tympanic membrane and thereby for close control of both sound pressure and static pressure directly in front of the tympanic membrane. Further the minimized volume of the AEC allowed for uniform pressure distribution with exclusion of canal effects. To confirm normal behavior of the temporal bones, physiological stapes vibrations in response to acoustic stimuli were examined and compared with the American Society for Testing and Materials (ASTM) standard F2504-05 (Philadelphia, 2005).

### 2.2. Static and dynamic pressure control

A pressure regulator PQ1 (AirCom Pneumatic GmbH, Germany) was used to generate static pressures in the ear canal. The static pressure was monitored in front of the tympanic membrane using an electronic pressure gauge HCLA0075B (First Sensor AG, Germany). Control of the static pressure was done via a LabView (version 2017, National Instruments, USA) interface with an accuracy of  $\pm 50$  Pa.

A loudspeaker (ER-2, Etymotic Research, USA) was placed in the AEC, and stepped-sine signals of 80 frequencies in a frequency range from 200 Hz to 8 kHz were delivered from a data acquisition device (DAQ) (NI-4431, National Instruments, USA) to the loudspeaker via an amplifier (RMX 850, QSC Audio Products LLC, USA). A microphone probe (ER-14C, Etymotic Research, USA) from a microphone (ER-7C, Etymotic Research, USA) was placed in the AEC with a distance of approximately 2–4 mm from the tympanic membrane in order to monitor the acoustic stimulation near the tympanic membrane. With an input of 1.25-V root mean square (RMS) to the loudspeaker, the sound pressure level (SPL) of the acoustic stimulation was in the range of 90–110 dB SPL in the considered frequency range.

### 2.3. Experimental procedure

A 3D-Laser Doppler vibrometer (LDV) system (CLV 3D-LDV, Polytec GmbH, Germany), which can measure three-dimensional components of vibrational motion of a point, was mounted on a robot arm (KR 16, KUKA, Germany), and was positioned such that an unobstructed view through the middle ear onto the desired measurement locations (the umbo and the stapes footplate center (SFC)) was obtained. The location of the SFC was estimated from microscopic view.

Small reflective beads of an approximately 50-micron diameter (Cospheric LLC, USA) were placed onto the measurement points to increase the signal to noise ratio. The velocities with three dimensional velocity components were measured at the umbo and the SFC with a sampling rate of 96 kS/s. The DAQ NI-4431 (National Instruments, USA) with Matlab scripts (Matlab versions 2018a, Mathworks) was used for recording of the velocity data.

The measurements were divided into four blocks, which consist of measurements under negative and positive static pressures in the AEC for each SFC (blocks 1 and 2, Table 1) and umbo (blocks 3 and 4). At each measurement block, the velocities of the umbo or the SFC were measured first under zero static pressure, and stepwise under negative or positive static ear-canal pressures. Before each block, the bones were preconditioned with a static pres-

**Table 1**

Four measurement blocks for each of flexible and flexibility-reduced conditions of the incudo-malleal joint (IMJ).

	Measurement point	Static pressure load (kPa)
<b>Block 1</b>	Stapes footplate center	0, -0.5, -1, -2
<b>Block 2</b>	(SFC)	0, +0.5, +1, +2
<b>Block 3</b>	Umbo	0, -0.5, -1, -2
<b>Block 4</b>		0, +0.5, +1, +2

sure of  $\pm 1$  kPa applied to the ear canal, as previous studies (e.g. Homma et al., 2010) indicated small changes in stapes and umbo velocities after application of positive or negative pressure to the ear canal.

After the measurements of all four blocks with the natural condition of the IMJ were completed, the flexibility of the IMJ was reduced and all four measurement blocks were repeated. The flexibility of the IMJ was reduced by injecting glue (Alleskleber Super Strong & Safe, UHU) into the joint capsule and applying a small amount of dental cement (Ionoglas Cem Extra, Harvard Dental International, USA) along the boundary of the IMJ. A method to reduce the flexibility of the IMJ was already established in our lab (see Fig.3 in Gerig et al., 2015), where it was shown that the method significantly reduces the relative motion between incus and malleus in a frequency range of 0.5 - 6 kHz by 10-15 dB (by 10 dB at 0.5 kHz and by 15 dB at 6 kHz). The reduced flexibility was not assessed by velocity measurements in this study, to shorten the measurement duration and thus to prevent the samples from drying effects. Instead, during the experiment the reduced flexibility of the IMJ was confirmed visually under the microscope by verifying that the malleus and the incus move as rigid body under static displacement (i.e. pressurized ear canal). Although the fixation procedure mentioned above does not indicate perfect fixation of the IMJ, we term this joint condition as “fixed (or immobilized)” in this study.

Deviation of the measurement point throughout the entire measurements on each temporal bone was minimized by applying the retroreflective beads to a small area near the targeted points (i.e., umbo and SFC) and aiming the laser beams to the center of the small area. The orientation of the temporal bone was fixed, and only a small adjustment in angular position of the LDV head was made to aim the laser beams to the measurement point. Therefore, deviation in angulation of the laser beams relative to the temporal bone through the entire measurements on each temporal bone was minimized as well. The temporal bones were kept hydrated during measurements by water mist and were immersed in a saline solution at least every 30 min, after which reflective beads were recoated to the area near the targeted points.

#### 2.4. Data analysis

All data processing and analysis was performed using customized Matlab scripts (Matlab versions 2018a and 2019a, Mathworks). The resultant of the measured three velocity components ( $v_x$ ,  $v_y$ , and  $v_z$ ) was calculated (Dobrev and Sim, 2018), and the transfer function (TF) was defined as the ratio of the resultant at the umbo or SFC to sound pressure in the ear canal. The effect of static pressure on the movement of the umbo or SFC was expressed with attenuation of the TF at the corresponding static pressure step relative to the TF at zero static pressure.

Paired *t*-tests followed by the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) was done to compare the attenuation relative to 0 kPa between the natural and fixed conditions of the IMJ, and between negative and positive static pressures. The statistical analysis was performed for 3 frequency bands (0.2–0.6 kHz; 0.6–1.4 kHz; 1.4–8 kHz). In each frequency band, the rela-

tive attenuation data were averaged through the specific frequency band. The difference was considered to be statistically significant in case that the adjusted *p*-value was less than 0.05.

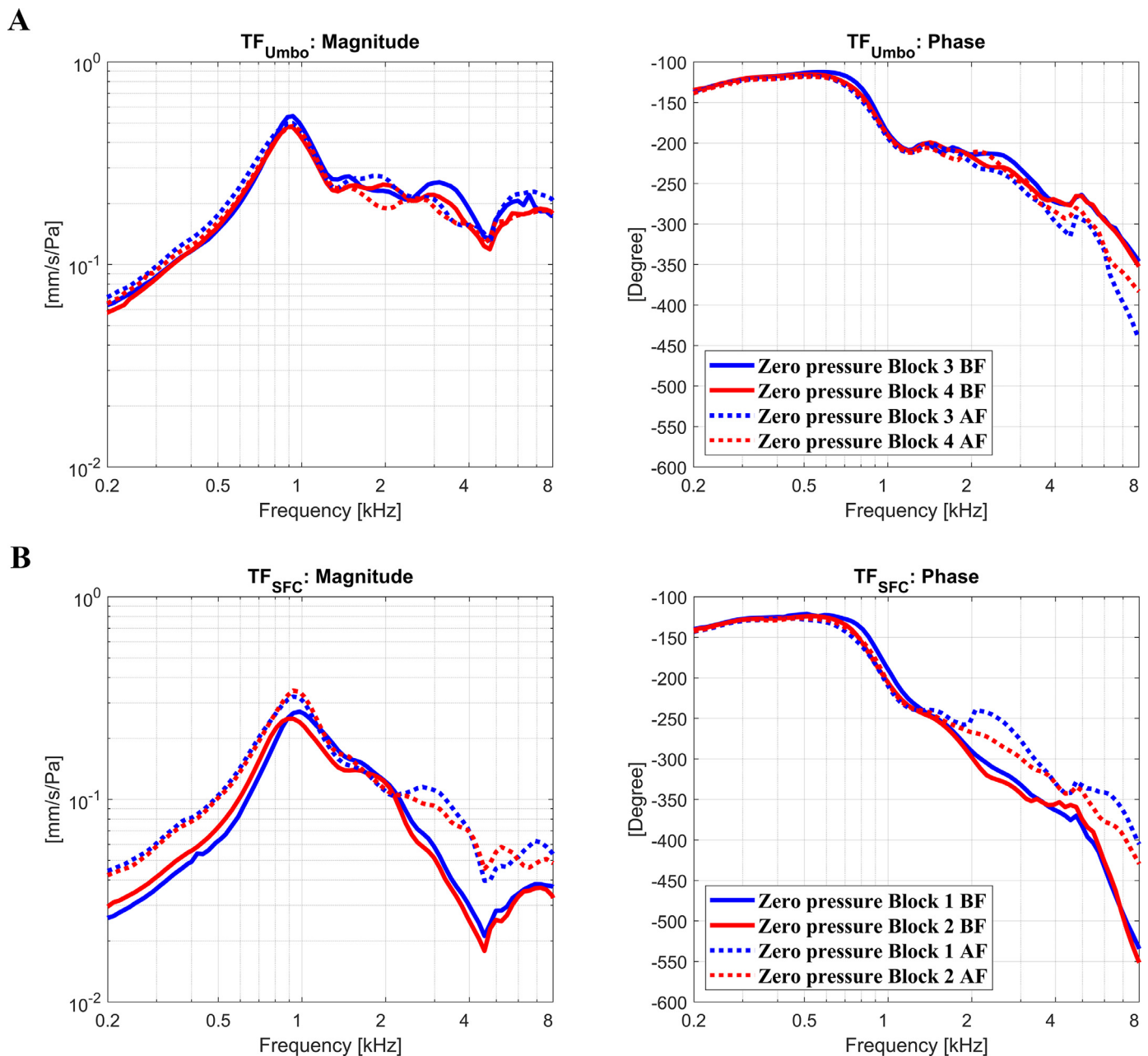
### 3. Results

Fig. 1 shows the TF of the umbo (A) and the SFC (B) under zero static pressure, for the four measurement blocks in Table 1, before (BF) and after fixation (AF) of the IMJ. The average values (geometric means for magnitudes and arithmetic means for phases) of the four temporal bones used in this study were plotted in the figure. In each temporal bone, the TFs were maintained almost the same between negative (block 1 and block 3) and positive (block 2 and block 4) static pressure series before and after the IMJ was fixed, indicating that applying static pressures does not cause considerable residual changes in mechanical properties of the middle ear structures. After the IMJ was fixed, the TF of the umbo did not show any considerable change whereas the TF of the SFC had slightly larger or similar magnitudes up to 2 kHz and larger magnitudes at higher frequencies. The larger magnitude of the TF of the stapes footplate at high frequencies above 2 kHz is in agreement with the finding in the study by Gerig et al., (2015). While the phase of the TFs of the umbo showed little change after fixation, the phase difference through the considered frequency range (0.2–8 kHz) became smaller for the TFs of the SFC.

Fig. 2 shows attenuations of the TFs of the umbo by static pressure loads before fixation of the IMJ. The thick solid lines indicate the mean values of the four temporal bones, and the shaded areas indicate the corresponding standard deviations. The TF decreased with increasing static pressure at frequencies below 1 kHz, where resonance of the middle-ear ossicular chain occurs. The attenuation of the TF at low frequencies was slightly larger for positive static pressures at  $\pm 0.5$  and  $\pm 1$  kPa, and was similar for positive (red in the figure) and negative (blue in the figure) static pressures at  $\pm 2$  kPa. The TF at low frequencies is reduced by 12–20 dB at  $\pm 2$  kPa. Statistically significant difference between the positive and negative pressure was observed only in the first (0.2–0.6 kHz) and the second (0.6–1.4 kHz, see Section 2.4) frequency bands at  $\pm 0.5$  kPa (the frequency range with the statistically significant difference is shown with \* in the figure). Similar attenuations of the umbo vibration at low frequencies caused by pressure difference across the tympanic membrane were observed in other works as well. The work by Murakami et al. (1997, represented as blue and red dots (•) in Fig. 2) showed that attenuations caused by positive static pressures in the ear canal were slightly larger than the attenuations caused by negative static pressures, at pressure magnitudes of 0.5, 1, and 2 kPa. Attenuation of the umbo vibration under  $\pm 2$ -kPa static pressures was investigated in the work by Homma et al. (2010, represented as x in Fig. 2) as well, and the attenuations had similar magnitudes for positive and negative static pressures. The phase change at each static pressure load relative to the phase at zero-static pressure load shows positive values around 1 kHz, indicating earlier responses of the umbo to acoustic stimuli near the resonance of the middle-ear ossicular chain.

Attenuations of the TFs of the SFC by static pressure loads are shown in Fig. 3. Generally, the trends in the relative attenuations of the TFs of the umbo were observed in the relative attenuations of the TFs of the SFC as well (i.e., attenuation of the TF at low frequencies with increasing static pressure in the ear canal). The attenuation under +0.5 kPa was slightly larger than the attenuation under -0.5 kPa whereas the attenuations under +1 and +2 kPa was similar to under attenuations under negative static pressures of the same magnitudes. Statistically significant difference between positive and negative static pressure loads was not observed in the first and second frequency bands. The similar trend was observed in the work by Murakami et al., but the attenuation observed in





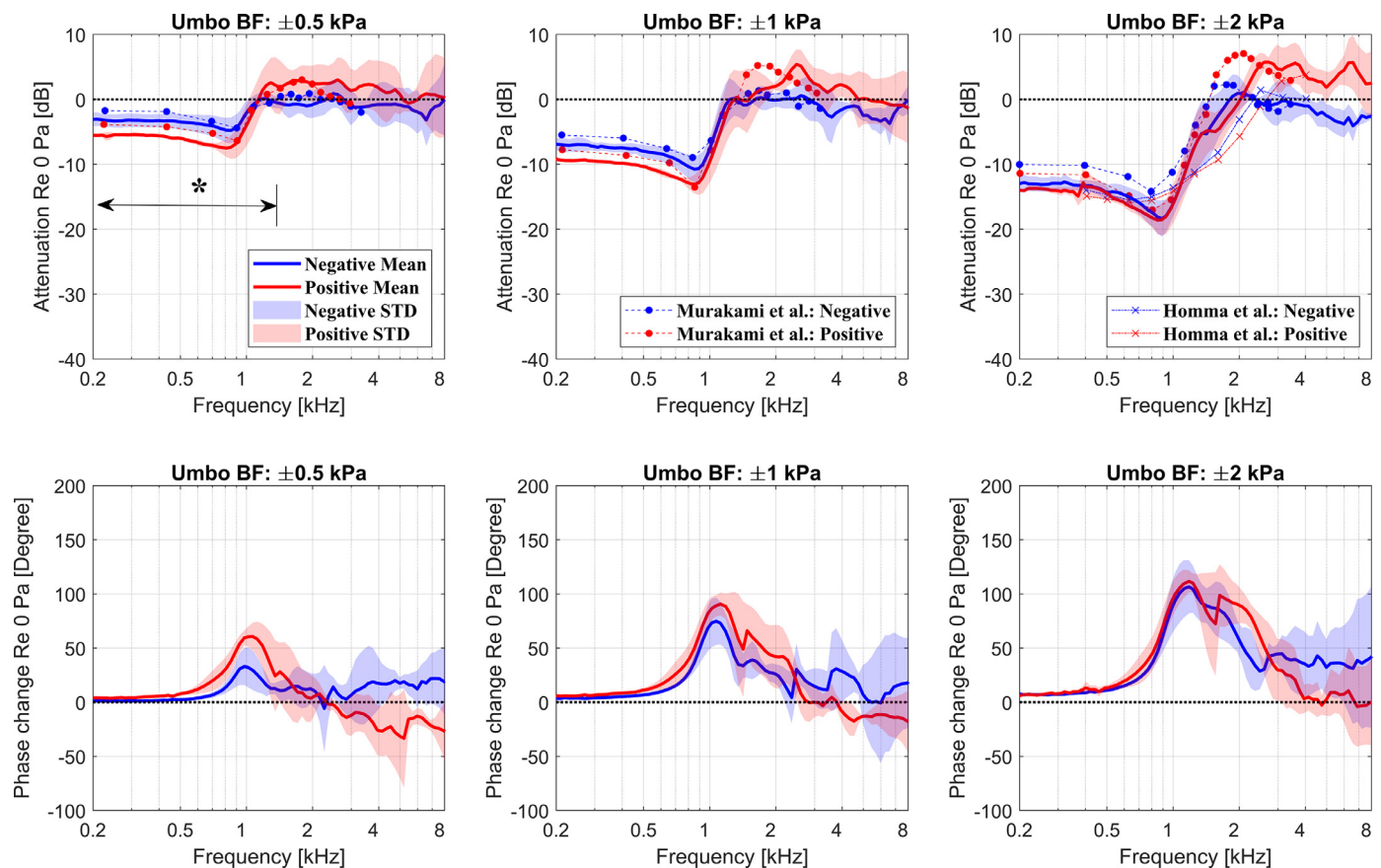
**Fig. 1.** Mean ( $n = 4$ ) transfer functions (TFs) of the umbo (A) and the stapes footplate center (SFC) (B), under zero static pressure through 4 measurement blocks in Table 1, before (solid line) and after (dotted line) fixation of the incudo-malleal joint (IMJ): magnitudes (left) and phases (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the present study was larger than the attenuation detected by Murakami et al. (dots (•) in Fig. 3). The attenuations at low frequencies (i.e., below 1 kHz) under  $\pm 2$ -kPa static pressures were similar to the corresponding data in the study by Homma et al. At frequencies above 1.4 kHz, the attenuation under -0.5 and -1 kPa was larger than the attenuation under +0.5 and +1 kPa, respectively. In this frequency band statistically significant difference between positive and negative pressures was observed only at  $\pm 0.5$  kPa. The phase difference with respect to the phase under zero pressure showed trends similar to the trends observed in the umbo vibration (i.e., positive phase differences relative to the phase with zero static pressure load around 1 kHz). The maximum phase change under  $\pm 2$  kPa was approximately  $100^\circ$  near 1 kHz.

After fixation of the IMJ, the attenuations of the umbo vibration under negative pressures were similar to the corresponding attenu-

ations before fixation whereas the attenuations of the umbo under positive static pressures increased (cf. Fig. 4). As a consequence, the attenuation of the TFs of the umbo below the middle-ear resonance under positive static pressures was larger by 6–7 dB than the corresponding attenuation with negative pressures, for  $\pm 0.5$ -,  $\pm 1$ -, and  $\pm 2$ -kPa static pressure loads. However, statistically significant differences were not obtained. The phase changes relative to the phase at zero static pressure load were similar to the relative phase changes before fixation of the IMJ.

Fig. 5 shows attenuations of the TFs of the SFC by static pressure loads after fixation of the IMJ. While the fixation of the IMJ affected the relative attenuations of the umbo only under positive static pressures, the relative attenuations of the SFC were changed for both positive and negative static pressure after fixation of the IMJ. The increase of the attenuation after fixation of the IMJ was



**Fig. 2.** Attenuations of the transfer functions (TFs) of the umbo by static pressure loads with the natural condition of the IMJ (i.e., before fixation (BF) of the IMJ): magnitude ratios (top) and phase changes (bottom). The solid lines are mean values and shaded area are the corresponding standard deviations with positive static pressure loads in red and negative pressure loads in blue ( $n = 4$ ). The corresponding data from literature (—•—: Murakami et al. 1997, —x—: Homma et al. 2010) are shown as well. \* indicates the frequency range where  $p$  values between the negative and positive static pressures are less than 0.05. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

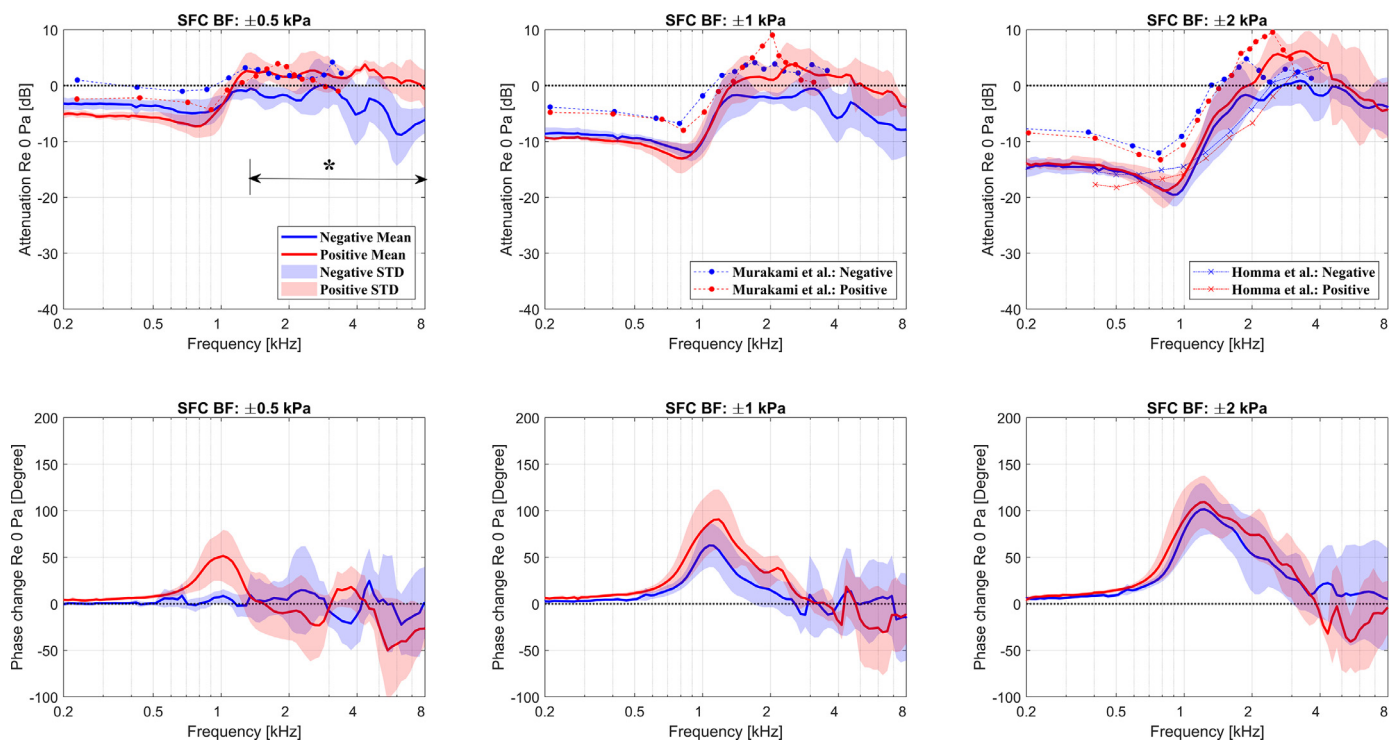
larger for positive pressures, resulting in 3.5–5 dB larger attenuation below the 1 kHz for positive static pressures. Statistically significant difference between positive and negative pressures was not obtained due to larger variations across samples.

To allow direct comparison between the natural (before fixation of the IMJ) and flexibility-reduced (after fixation of the IMJ) conditions of the IMJ, the ratios of the attenuation after fixation relative to the attenuation before fixation are plotted in Fig. 6. As shown in the figure, considerable changes in the attenuation after fixation of the IMJ at low frequencies occurred with positive static pressure loads for the umbo (Fig. 6A), and with both positive and negative pressure loads for the SFC (Fig. 6B). With a pressure load of +2 kPa, the change in the attenuation after the fixation of the IMJ reached ~6 dB for the umbo and ~11 dB for the SFC. The attenuation of the TFs of the SFC increased by ~7 dB for a pressure load of -2 kPa. Statistically significant change caused by fixation of the IMJ was observed for the umbo with positive pressure loads of +0.5 and +1 kPa, and for the SFC with positive pressure loads of +0.5, +1, and +2 kPa. The  $p$ -value for the umbo with a positive pressure loads of +2 kPa ( $\approx 0.059$ ) was near the border of statistical significance.

#### 4. Discussion

Cadaveric human temporal bones are subject to immediate and slow post-mortem changes, as discussed in our previous study (Gerig et al., 2015). The immediate post-mortem changes are believed to affect the middle ear mechanics insignificantly (von

Békésy, 1960). With the use of frozen temporal bones, effects of slow post-mortem changes were inescapable in this study, but only temporal bones showing normal stapes responses to acoustic stimuli within the ASTM F2504-05 standard (2005) were included in the present study. Gerig et al. (2015) showed that drying of temporal bones causes attenuation at lower frequencies and an increase of the middle-ear sound transmission at higher frequencies already after 30 min without being moistened. These changes in the middle-ear sound transmission are reversible by immersion of the temporal bone in a saline solution. In this study, to minimize changes of mechanical properties of the middle ear during the measurements, the sample was moisturized by a water spray every 10 min throughout the experiment. Moreover, the sample was rehydrated in a saline solution every 30 min, after which the retro-reflective beads were reapplied around the measurement locations (i.e., SFC and umbo). The measurement locations of the SFC and the umbo were not maintained exactly the same before and after immersion of the sample into saline, however, similar locations were searched in the magnified view through the camera, which was mounted on the LDV system. The magnitudes of static pressure loads used in this study are similar to the magnitudes of static pressure loads in clinical tympanometry. Therefore, the mechanical properties of the middle-ear tissues are presumed not to be changed with such magnitudes of static pressure loads in live human subjects. Murakami et al. (1997) and Homma et al. (2010) applied static pressure loads of magnitudes similar to the magnitudes of static pressure loads of the present study, showing that considerable irreversible changes in mechanical properties were not ob-



**Fig. 3.** Attenuations of the transfer functions (TFs) of the stapes footplate center (SFC) by static pressure loads with the natural condition of the IMJ (i.e., before fixation (BF) of the IMJ): magnitude ratios (top) and phase changes (bottom). The solid lines are mean values and shaded area are the corresponding standard deviations with positive static pressure loads in red and negative pressure loads in blue ( $n = 4$ ). The corresponding data from literature (—•—: Murakami et al. 1997, —x—: Homma et al. 2010) are shown as well. \* indicates the frequency range where  $p$  values between the negative and positive static pressures are less than 0.05. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

served in cadaveric temporal bones. To verify that no considerable change was caused by loading, hydration or post mortem effects over the entire experiment, measurements of vibrational motion under zero static pressure were repeated at the beginning of each measurement block (cf. Table 1). The repeatability of the measurements shown in Fig. 1 (between the blue and red curves) indicates no considerable irreversible changes in mechanical properties.

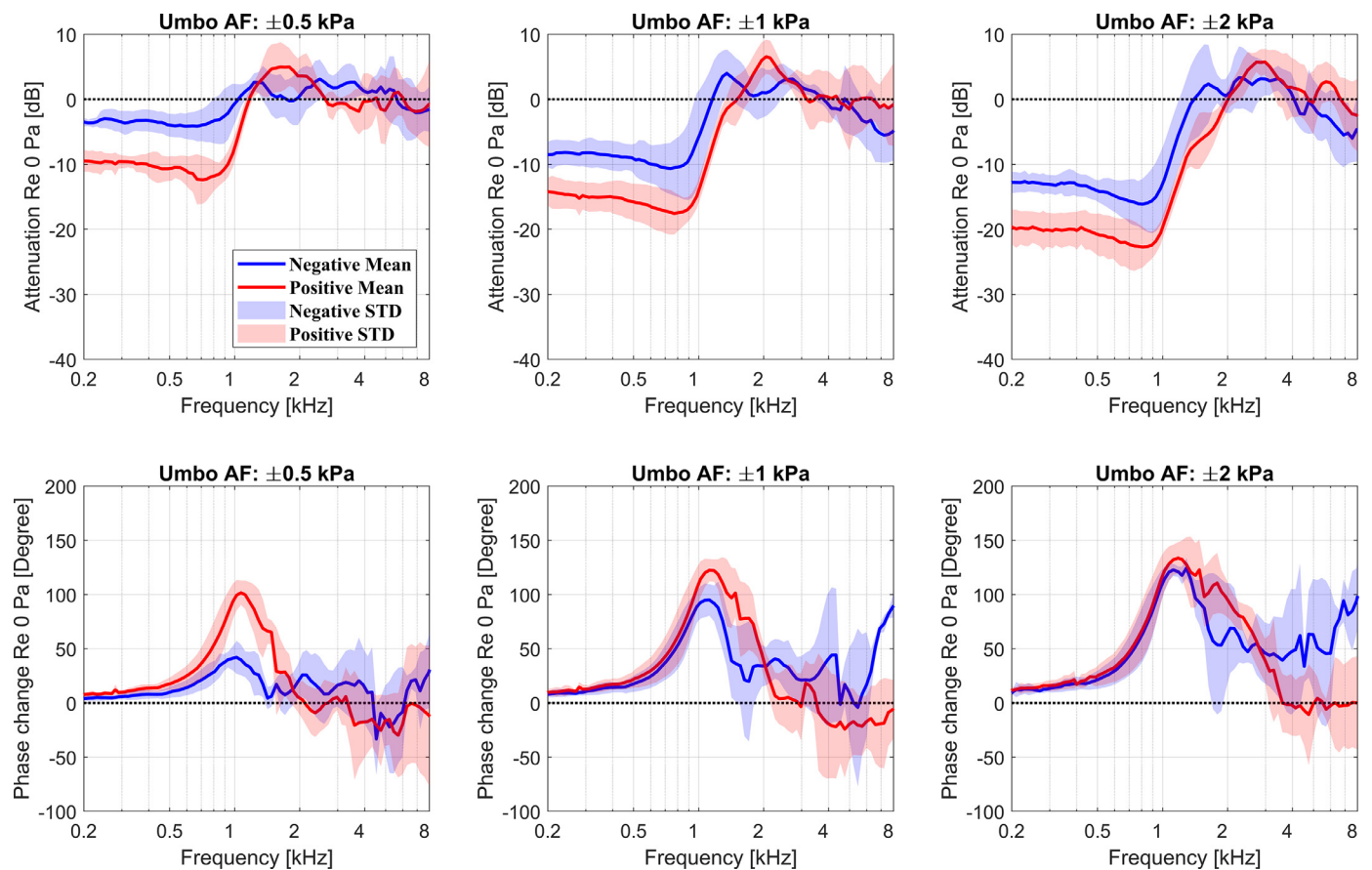
While results from measurements on only four temporal bones were considered in this study, more than 10 temporal bones were used to extract samples in agreement with the ASTM standard and to establish a robust measurement procedure which allowed immersion of the samples into the saline solution every 30 min as well. In detail, three bones were used to set up a concise measurement protocol which allowed simultaneously for a tight seal of the AEC, a tight pressure control at the TM, quick fixation of the IMJ, and minimal drying effects with immersion of the sample into saline every 30 min. Two bones were excluded due to damages during preparation or measurement, and two other bones were excluded due to not complying to the ASTM standard. While the results from only four samples are reported, consideration of the considered measurement protocol allowed for the low inter-sample variability displayed in this study and the statistical tests conducted indicated a good reliability of the presented results.

The static pressure range in this study was chosen such that it represents changes in healthy ears with a functioning eustachian tube, as the eustachian tube passively opens at a differential pressure of c.a. 2 kPa, leading to pressure equalisation (Mirza and Richardson, 2005).

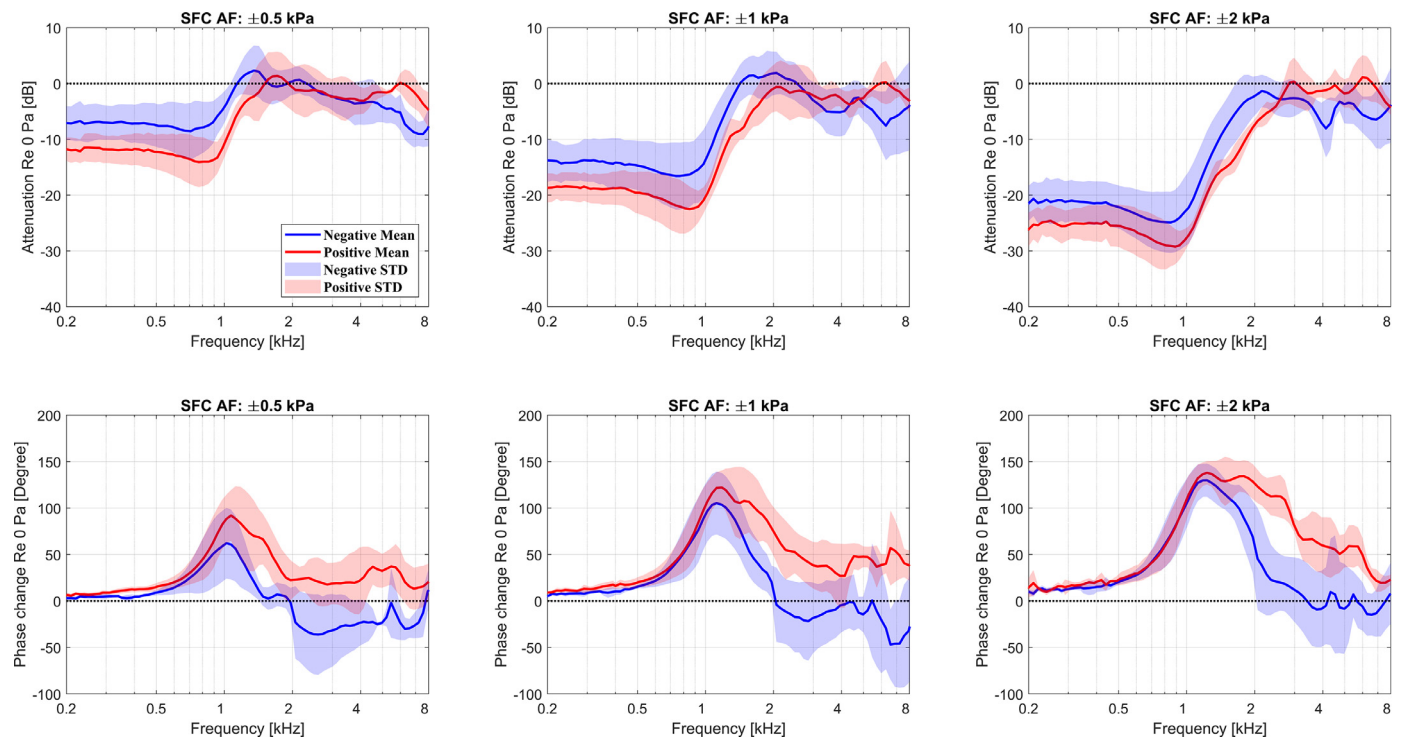
The measurements of the middle ear vibration with the natural IMJ (i.e., before fixation) under static pressures showed two main trends as observed in previous studies (Murakami et al. 1997; Homma et al., 2010). These are: 1) attenuation of umbo and stapes vibration below the first resonance frequency of the

middle ear and 2) slightly larger attenuation under positive static pressures when the magnitude of the static pressure is small. Based on finite-element model simulations of the human middle ear, Wang et al. (2007) proposed two mechanisms for the decrease of middle-ear vibration in the stiffness-dominant frequency range (i.e., low frequencies below the first middle-ear resonance frequency) caused by static pressure difference across the tympanic membrane; i) changes of elastic moduli of soft tissues and ii) geometrical variation of the middle ear structures. In their work, effects of the geometrical variation were defined effects by changes of the shape of the tympanic membrane and changes of location and orientation of the ligaments and tendons, without considering changes of elastic moduli of soft tissues caused by elongation or contraction of the soft tissues. Their simulations showed that attenuation under positive static pressures in the ear canal is larger than attenuation under negative static pressures due to the different effects of the geometrical variation. According to their model, geometrical variation of the middle-ear structures induced by positive static pressures generates higher attenuation of umbo and stapes vibrations than geometrical variation induced by negative static pressures. Another finite-element model simulation by Ihrle et al. (2013) provided a similar explanation about the attenuation of middle-ear vibration under static pressures. They used terms of “geometrical nonlinearity” and “nonlinearity in material properties” of the middle-ear structures to explain two mechanisms for attenuation of middle-ear sound transmission under static pressures. They also proposed that geometrical changes, which include changes of the shape of the tympanic membrane and changes of relative location and orientation of the ligaments and tendons, contribute to higher attenuation of middle-ear sound transmission under positive static pressure in the ear canal. Especially, a geometrical change of the tympanic membrane was emphasized in their work. According to their work, inward

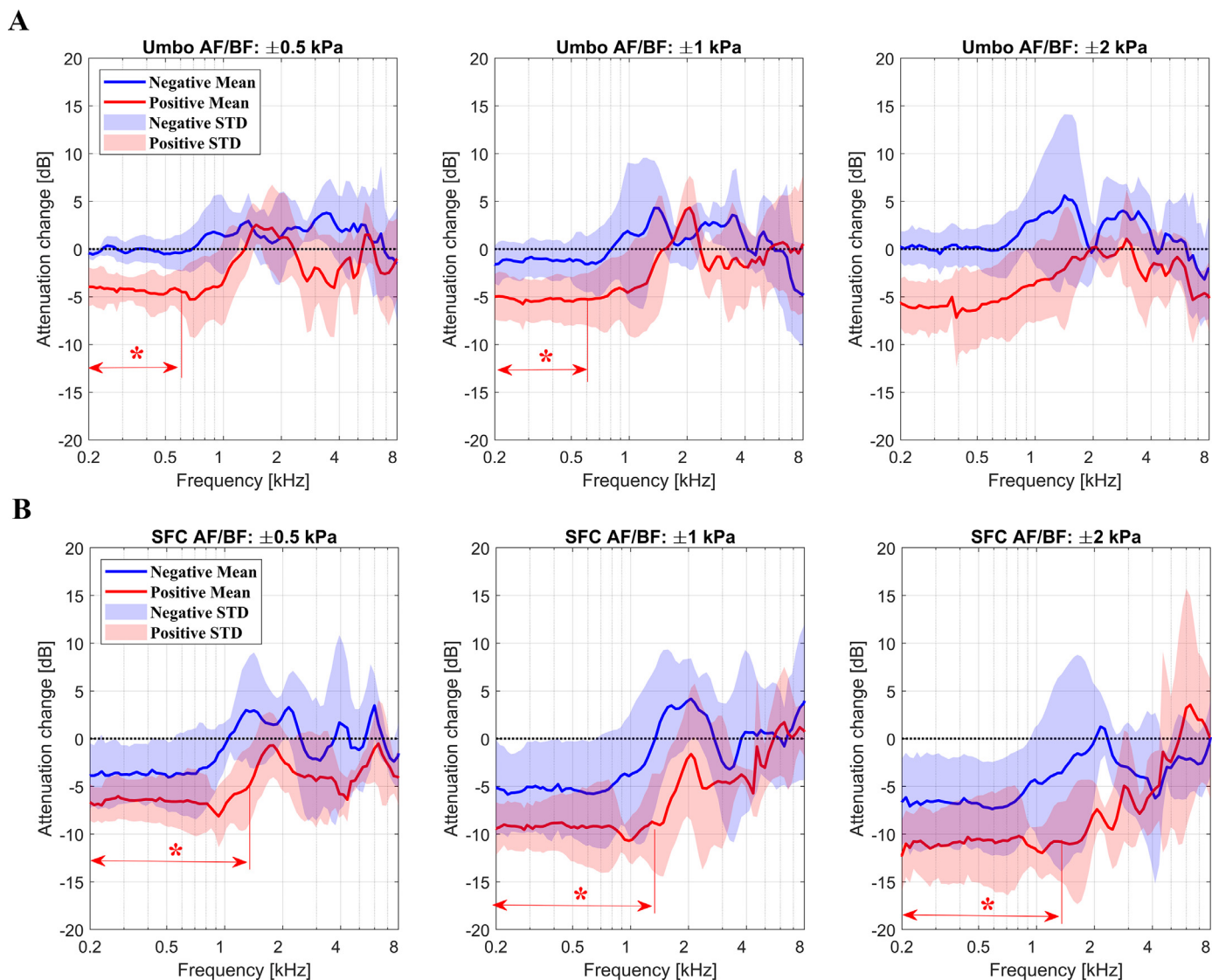




**Fig. 4.** Attenuations of the transfer functions (TFs) of the umbo by static pressure loads after fixation (AF) of the IMJ, at each static pressure load over the transfer function at zero static pressure load: magnitude ratios (top) and phase difference (bottom). The solid lines are mean values and shaded area are the corresponding standard deviations with positive static pressure loads in red and negative pressure loads in blue ( $n = 4$ ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 5.** Attenuations of the transfer functions (TFs) of the stapes footplate center (SFC) by static pressure loads after fixation (AF) of the IMJ: magnitude ratios (top) and phase changes (bottom). The solid lines are mean values and shaded area are the corresponding standard deviations with positive static pressure loads in red and negative pressure loads in blue ( $n = 4$ ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 6.** Ratios of the attenuation after fixation relative to the attenuation before fixation: umbo (A) and stapes footplate center (SFC) (B). The solid lines are mean values and shaded area are the corresponding standard deviations with positive static pressure loads in red and negative pressure loads in blue ( $n = 4$ ). \* indicates the frequency range where attenuation change after fixation of the IMJ is statistically significant ( $p < 0.05$ ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

movement of the tympanic membrane under positive static pressure in the ear canal makes the cone depth larger, resulting in geometrical hardening of the tympanic membrane.

Though general trends observed in this study are similar to the trends reported in Murakami et al., the attenuations in vibration of the umbo and stapes observed in this study was larger than the corresponding attenuations in Murakami et al., especially for stapes vibrations under high static pressures. In the following, we will suggest several possible reasons that may explain the discrepancy between the two studies. First, while the static pressure was applied in the ear canal in this study, the middle-ear cavity was pressurized in Murakami et al. The different types of pressure loading may cause different relative movement between the malleus and the incus at the IMJ, resulting in different geometrical changes of the incus and stapes and different stress levels at the annular ligament of the stapes. Homma et al. (2010), who used the same type of pressure loading as of the present study, reported similar magnitudes of attenuations of umbo and stapes vibration as we have observed. Second, in contrast to Murakami et al., where only one-dimensional vibration of the stapes head was investigated, we

measured the three-dimensional vibration components of the SFC. It is known that stapes vibration in the three-dimensional space contains a piston-like translational component and two rocking-like rotational components (Hato et al., 2003; Sim et al., 2010). While vibration of the SFC is determined mainly by the piston-like components, vibration of the stapes head is determined by a combination of the piston-like component and the rocking-like components. Consequently, with presence of the rocking-like components, the direction of stapes head vibration varies in the frequency domain and therefore the one-dimensional measurement of the stapes head vibration is dependent on the measurement direction. Third, the static pressure in the ear canal was quantified using an electronic pressure gauge that measured the static pressure close to the tympanic membrane in the present study whereas Murakami et al. used the pressure sensor of the pressure controller to quantify the static pressure in the middle-ear cavity. Such an approach is only valid if no or small leakages occur in the pneumatic system consisting of regulator, connecting pressure line and middle-ear cavity. In the case that leakage flow prevails in the system, a pressure drop is induced by viscous loss of

the air flow. On certain temporal bones we could observe differences larger than 10% between the pressure readings of the pressure gauge at the tympanic membrane and inside the controller. Another possible reason to explain the larger attenuation in the present study may be the use of frozen temporal bones compared to use of fresh samples in Murakami et al. While previous studies (e.g., Rosowski et al., 1990) showed comparable middle-ear input impedance and acoustical response of an unloaded middle ear for frozen and fresh temporal bones, their difference under static pressure loads have not been investigated.

The main effect of IMJ fixation was a larger attenuation of the umbo vibration under the positive static pressures and of the SFC vibration under both positive and negative pressures (see Fig. 6). The attenuation of the SFC vibration after IMJ fixation was more pronounced for positive static pressures. The larger attenuation after IMJ fixation may be explained by a larger displacements of the incus and stapes under static pressures with the fixed condition of the IMJ. Works by Hüttenbrink (1988, 1997) showed that the flexibility of the IMJ in the human middle ear decouples the incus and stapes from large displacements of the tympanic membrane and the malleus that are induced by static pressure differences across the tympanic membrane. According to his measurements, the average ratio of inward/outward displacement between the umbo and the stapes under 4 kPa was 21:1 in the normal human middle ear. However, when the IMJ was ankylosed, movement of the stapes became larger even though movement of the umbo decreased, resulting in the average ratio between the umbo and the stapes of 4.2:1. Therefore, with the reduced flexibility of the IMJ, larger displacements of the incus and stapes are expected, resulting in higher stress levels in ligaments and tendons of the incus and the stapes. As it was observed in this study that the TFs of the SFC were more affected by the fixation of the IMJ than the TFs of the umbo, it is presumed that the fixation of the IMJ mainly increases the stress level of the stapes annular ligament due to larger preloads on the annular ligament caused by the larger displacement of the stapes. However, it is difficult to explain why the fixation of the IMJ have larger effect on vibrations of the umbo and SFC under positive pressures than the corresponding vibrations under negative pressures. As discussed above, Wang et al. (2007) and Ihrle et al. (2013), proposed that both geometrical change of the middle-ear structures and change of elastic moduli of the soft tissues are related to attenuation of the middle-ear vibration under static pressure difference across the tympanic membrane. Reinforcement of the two mechanisms by larger displacements of the incus and the stapes with the flexibility-reduced IMJ would be different for positive and negative pressures. The larger additional attenuation induced by the reduced flexibility of the IMJ for positive static pressures can be due to either more reinforcement of soft-tissue stiffening under positive static pressures, more favorable effects of the geometrical change on middle-ear sound transmission under negative static pressures, or combination of both. More information on these mechanisms with varying flexibility of the IMJ is expected to be revealed with simulation of a comprehensive middle-ear model with a delicate description of the flexibility of the IMJ in the future.

Though the flexibility of the IMJ performs important roles to protect inner-ear structures and reduce attenuation of middle-ear sound transmission under changes of surrounding static pressure, current clinical diagnosis procedures does not identify stiffening of the IMJ separately. According to the results of this study (Fig. 4 and 6A), the relative attenuations of the TFs of the umbo shows clear nonsymmetric behavior between the positive and negative static pressures with fixation of the IMJ. This indicates that the admittance curve from tympanometry, which is measured with pressure loads of 226 Hz, may show a nonsymmetric feature between the positive and negative static pressures in the case that the IMJ

is stiffened and thus the nonsymmetric curve in tympanometry may identify stiffening of the IMJ. Since the nonsymmetric admittance curve in tympanometry might be caused by other pathological conditions as well, further investigation will be necessary to confirm the diagnostic utility.

The presented results revealed that attenuations of middle-ear sound transmission under static pressure loads were larger with a stiffened IMJ and therefore, the flexible IMJ allowed for better middle-ear sound transmission under static pressure loads and thus adaptive function to surrounding static pressure changes. However, this adaptive function of the flexible IMJ, as well as its protective function, have been little considered for reconstruction of the middle ears. Stoppe et al. (2018) investigated advantages of a total ossicular replacement prosthesis with a silicone coated ball and socket joint, which aimed at mimicking the flexible IMJ. Under positive middle-ear cavity pressures (which corresponds to the negative EC static pressure of this study), dislocation of the prosthesis occurred regardless of existence of the ball and joint, leading to an interruption of the ossicular chain. However, under negative middle-ear cavity pressure (which corresponds to the positive EC static pressure of this study), they found advantages of the ball-joint prosthesis for middle-ear sound transmission in comparison to the traditional rigid prostheses, which is consistent to the results of the this study. The results presented in this article suggest that adaptive function as well as the protective function of the flexible IMJ need to be considered for design of middle-ear prostheses.

## 5. Conclusion

Effects of the flexibility of the incudo-malleal joint (IMJ) on middle-ear sound transmission under static pressures were investigated from experiments in cadaveric human temporal bones. Vibrational motions of the umbo and the stapes footplate center in responses to acoustic stimulation were measured under static pressures in the ear canal, with the natural and flexibility-reduced conditions of the IMJ.

The results of this study showed that attenuations of middle-ear vibrational motion induced by static pressure loads were enhanced by reducing flexibility of the IMJ, for the umbo under positive static pressures and the stapes footplate center under both positive and negative static pressures. The findings of this study indicate a further advantage or possible evolutionary drive towards the flexible joint by allowing better sound transmission through the middle ear under static pressure differences across the tympanic membrane. The results of this study are expected to be used for diagnosis of stiffening of the IMJ and for design of the middle-ear prostheses.

## CRediT authorship contribution statement

**Birthe Warnholtz:** Methodology, Software, Formal analysis, Investigation, Writing – original draft. **Merlin Schär:** Investigation, Investigation, Writing – review & editing. **Benjamin Sackmann:** Methodology, Resources. **Michael Lauxmann:** Methodology, Writing – review & editing. **Michail Chatzimichalis:** Methodology, Investigation. **Lukas Prochazka:** Methodology, Resources, Writing – review & editing. **Ivo Dobrev:** Methodology, Software, Investigation. **Alexander M. Huber:** Conceptualization, Supervision, Project administration, Funding acquisition. **Jae Hoon Sim:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration.

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